AMENDMENTS TO THE SPECIFICATION

Please amend the specification as follows:

Please delete the previously filed Sequence Listing and insert the substitute Sequence Listing filed herewith.

Replace paragraph [0048]:

"[0048] The invention described herein produces recombinant fusion proteins consisting of a unique GBP (Brown, Nat. Biotechnol. 15:269-272, 1997) consisting of 7 repeats of the 14 amino acid sequence, Met-His-Gly-Lys-Thr-Gln-Ala-Thr-Ser-Gly-Thr-Ile-Gln-Ser, and any desired polypeptide specifying activity, binding such fusion protein to a gold surface thereby introducing functionality to the surface. The invention provides the following improvements compared to existing methods:"

with,

--[0048] The invention described herein produces recombinant fusion proteins consisting of a unique GBP (Brown, Nat. Biotechnol. 15:269-272, 1997) consisting of 7 repeats of the 14 amino acid sequence, Met-His-Gly-Lys-Thr-Gln-Ala-Thr-Ser-Gly-Thr-Ile-Gln-Ser (SEQ ID NO:17), and any desired polypeptide specifying activity, binding such fusion protein to a gold surface thereby introducing functionality to the surface. The invention provides the following improvements compared to existing methods: --

Replace paragraph [0067]:

"[0067] Construction of the expression plasmid for Protein A-GBP fusion protein. The plasmid pSB3053 obtained from S. Brown (Brown, Nat. Biotechnol. 15:269-272, 1997) was used as the

source of the GBP fragment containing seven repeats of the peptide MHGKTQATSGTIQS. Upon DNA sequencing it was found that the last repeat carried a substitution of the threonine residue in the fifth position for an isoleucine. All the fusion proteins constructed in this work have this substitution."

with,

--[0067] Construction of the expression plasmid for Protein A-GBP fusion protein. The plasmid pSB3053 obtained from S. Brown (Brown, Nat. Biotechnol. 15:269-272, 1997) was used as the source of the GBP fragment containing seven repeats of the peptide MHGKTQATSGTIQS (SEQ ID NO:17). Upon DNA sequencing it was found that the last repeat carried a substitution of the threonine residue in the fifth position for an isoleucine. All the fusion proteins constructed in this work have this substitution. --

Replace paragraph [0068]:

"[0068] An EcoR I-Xho I fragment encompassing the GBP coding sequence was excised from pSB3053 and adapted at the 3' end to include coding triplets for the amino acids EGP and a stop codon. Oligonucleotides BH3 (5' TCG AGG GTC CGT AAT A 3') and BH4 (5' AGC TTA TTA CGG ACC C 3') were annealed to obtain an adaptor with Xho I and Hind III cohesive ends. The EcoRI-Xho I GBP containing fragment and the adaptor were assembled in pUC18 and cut with EcoR I and Hind III in a three-part ligation to obtain plasmid pBHI-1. The Bsl I-Hind III fragment from pBHI-1 carrying the GBP coding sequence was adapted at its 5' end to include an in-frame linker sequence with an Asn-Gly hydroxylamine sensitive cleavage site. Oligonucleotides BH1 (5' CTG GTA GTG GCA ATG GTC ATA TGC 3') and BH2 (5' TAT GAC CAT TGC CAC TAC CAG AGC T 3') were annealed to obtain an adaptor with Sac I and

Bsl I cohesive ends. The adaptor also incorporates an Nde I site at the methionine codon of the first GBP repeat for ease of adaptation of the GBP fragment with any desired in-frame sequence. Plasmid pBHI-2 was generated with the Bsl I GBP fragment this adaptor and pUC19 linearized with Sac I and Hind III, in a three-part ligation. The nucleotide sequence of the Sac I-Hind III, double-adapted GBP fragment was confirmed by DNA sequencing. The Sac I-Hind III fragment from pBHI-2 was cloned between the Sac I and Hind III sites of pEZZ18 (Amersham) for an in-frame fusion with the two Z domains of staphylococcal Protein A (Nilsson, et al., Protein Eng 1:107-113, 1987) to obtain plasmid pBHI-3. The final expression plasmid for the cytoplasmic production of the His-tagged fusion protein was constructed by ligating the Protein A-GBP containing Fsp I-Hind III fragment from pBHI-3 and a short adaptor sequence formed by oligonucleotides BH11 and BH12 (5' GAT CCG GTT CTG GTG C.sub.3' and 5' GCA CCA GAA CCG 3', respectively) into pQE-80L (Qiagen, Inc) cut with BamH I and Hind III. The resulting plasmid, called pPA-GBP, is depicted in FIG. 2. The nucleotide sequence of the encoded fusion protein was confirmed by DNA sequencing. The complete DNA sequence of pPA-GBP and the amino acid sequence of the fusion protein appear in the Sequence Listing section at the end of this document."

with,

--[0068] An EcoR I-Xho I fragment encompassing the GBP coding sequence was excised from pSB3053 and adapted at the 3' end to include coding triplets for the amino acids EGP and a stop codon. Oligonucleotides BH3 (5' TCG AGG GTC CGT AAT A 3' (SEQ ID NO:18)) and BH4 (5' AGC TTA TTA CGG ACC C 3' (SEQ ID.NO:19)) were annealed to obtain an adaptor with Xho I and Hind III cohesive ends. The EcoRI-Xho I GBP containing fragment and the adaptor

were assembled in pUC18 and cut with EcoR I and Hind III in a three-part ligation to obtain plasmid pBHI-1. The Bsl I-Hind III fragment from pBHI-1 carrying the GBP coding sequence was adapted at its 5' end to include an in-frame linker sequence with an Asn-Gly hydroxylamine sensitive cleavage site. Oligonucleotides BH1 (5' CTG GTA GTG GCA ATG GTC ATA TGC 3' (SEQ ID NO:20)) and BH2 (5' TAT GAC CAT TGC CAC TAC CAG AGC T 3' (SEQ ID NO:21)) were annealed to obtain an adaptor with Sac I and Bsl I cohesive ends. The adaptor also incorporates an Nde I site at the methionine codon of the first GBP repeat for ease of adaptation of the GBP fragment with any desired in-frame sequence. Plasmid pBHI-2 was generated with the Bsl I GBP fragment this adaptor and pUC19 linearized with Sac I and Hind III, in a three-part ligation. The nucleotide sequence of the Sac I-Hind III, double-adapted GBP fragment was confirmed by DNA sequencing. The Sac I-Hind III fragment from pBHI-2 was cloned between the Sac I and Hind III sites of pEZZ18 (Amersham) for an in-frame fusion with the two Z domains of staphylococcal Protein A (Nilsson, et al., Protein Eng 1:107-113, 1987) to obtain plasmid pBHI-3. The final expression plasmid for the cytoplasmic production of the His-tagged fusion protein was constructed by ligating the Protein A-GBP containing Fsp I-Hind III fragment from pBHI-3 and a short adaptor sequence formed by oligonucleotides BH11 and BH12 (5' GAT CCG GTT CTG GTG C 3' (SEQ ID NO:22) and 5' GCA CCA GAA CCG 3' (SEQ ID NO:23), respectively) into pQE-80L (Qiagen, Inc) cut with BamH I and Hind III. The resulting plasmid, called pPA-GBP, is depicted in FIG. 2. The nucleotide sequence of the encoded fusion protein was confirmed by DNA sequencing. The complete DNA sequence of pPA-GBP and the amino acid sequence of the fusion protein appear in the Sequence Listing section at the end of this document.--

Replace paragraph [0069]:

[0069] Construction of the expression plasmid for Streptavidin-GBP fusion protein. The coding sequence for core streptavidin residues 13-139 of the mature polypeptide (Sano, et al., J Biol Chem 270:28204-28209, 1995) was derived from a pUC18-based plasmid obtained from Dr. P. Stayton (Chilkoti et al., Proc Natl Acad Sci USA 92:1754-1758, 1995). A Sac I restriction site was engineered into the coding sequence to allow fusions to the shortened version of streptavidin, residues 13-133 (Sano, et al., J Biol Chem 270:28204-28209, 1995). For this, an EcoR 1-Mlu I fragment encoding the partial core streptavidin sequence was linked to an adaptor with Mlu I and Hind III cohesive ends (formed using oligo pairs BH7/BH8, 5' CGC GTG GAA ATC CAC CCT GGT TGG TCA 3'/5' GTG TCG TGA CCA ACC AGG GTG GAT TTC CA 3' and BH9/BH10 5' CGA CAC CTT CAC CAA AGT TTC GAG CTC 3'/5' AGC TTG AGC TCG AAA CTT TGG TGA AG 3') and inserted into pUC18 cut with EcoR I and Hind III to yield pBHI-5. The nucleotide sequence of the total EcoR I-Hind III insert in pBHI-5 was confirmed by DNA sequencing.

with,

--[0069] Construction of the expression plasmid for Streptavidin-GBP fusion protein. The coding sequence for core streptavidin residues 13-139 of the mature polypeptide (Sano, et al., J Biol Chem 270:28204-28209, 1995) was derived from a pUC18-based plasmid obtained from Dr. P. Stayton (Chilkoti et al., Proc Natl Acad Sci USA 92:1754-1758, 1995). A Sac I restriction site was engineered into the coding sequence to allow fusions to the shortened version of streptavidin, residues 13-133 (Sano, et al., J Biol Chem 270:28204-28209, 1995). For this, an EcoR 1-Mlu I fragment encoding the partial core streptavidin sequence was linked to an adaptor

with Mlu I and Hind III cohesive ends (formed using oligo pairs BH7/BH8, 5' CGC GTG GAA ATC CAC CCT GGT TGG TCA 3' (SEQ ID NO:24)/5' GTG TCG TGA CCA ACC AGG GTG GAT TTC CA 3' (SEQ ID NO:25) and BH9/BH10 5' CGA CAC CTT CAC CAA AGT TTC GAG CTC 3' (SEQ ID NO:26)/5' AGC TTG AGC TCG AAA CTT TGG TGA AG 3' (SEQ ID NO:27)) and inserted into pUC18 cut with EcoR I and Hind III to yield pBHI-5. The nucleotide sequence of the total EcoR I-Hind III insert in pBHI-5 was confirmed by DNA sequencing.--

Replace paragraph [0070]:

"[0070] Using an Nde I site present at the initiating methionine of the adapted core streptavidin sequence in pBHI-5, the Nde I-Hind III fragment encoding core streptavidin was cloned into the expression vector pQE-80L (Qiagen, Inc), digested with BamH I and Hind III. A short adaptor sequence with BamH I and Nde I cohesive ends, formed with the oligo pair BH17/BH18 (5' GAT CCG GTT CTG GTG GCC A 3'/5' TAT GGC CAC CAG AAC CG 3') was used for linking."

with,

--[0070] Using an Nde I site present at the initiating methionine of the adapted core streptavidin sequence in pBHI-5, the Nde I-Hind III fragment encoding core streptavidin was cloned into the expression vector pQE-80L (Qiagen, Inc), digested with BamH I and Hind III. A short adaptor sequence with BamH I and Nde I cohesive ends, formed with the oligo pair BH17/BH18 (5' GAT CCG GTT CTG GTG GCC A 3' (SEQ ID NO:28)/5' TAT GGC CAC CAG AAC CG 3' (SEQ ID NO:29)) was used for linking.--

Replace paragraph [0071:

"[0071] The resulting plasmid called pBHI-7 can produce a N-terminal His-tagged core

streptavidin molecule residues 13-133, ending with the added amino acid residues SSSSILS. To express the His-tagged core streptavidin-GBP fusion protein, the engineered Sac I site in the core streptavidin sequence (see above) was utilized to link the Sac I-Hind III GBP encoding fragment from pBHI-2 to generate the expression plasmid pStreptavidin-GBP which has the basic backbone of the expression vector pQE 80L (Qiagen, Inc). The plasmid map, pStreptavidin-GBP is depicted in FIG. 3 and relevant DNA and amino acid sequences appear in the Sequence Listing section at the end of this document."

with

--[0071] The resulting plasmid called pBHI-7 can produce a N-terminal His-tagged core streptavidin molecule residues 13-133, ending with the added amino acid residues SSSSILS (SEQ ID NO:30). To express the His-tagged core streptavidin-GBP fusion protein, the engineered Sac I site in the core streptavidin sequence (see above) was utilized to link the Sac I-Hind III GBP encoding fragment from pBHI-2 to generate the expression plasmid pStreptavidin-GBP which has the basic backbone of the expression vector pQE 80L (Qiagen, Inc). The plasmid map, pStreptavidin-GBP is depicted in FIG. 3 and relevant DNA and amino acid sequences appear in the Sequence Listing section at the end of this document.--